

## An Environment for Development

The issue of how chemicals and metals in the environment affect human health continues to gain attention and attract more research dollars. However, most of the research being conducted on the issue tends to focus on adults, with precious few studies examining how exposure to these substances during development affects children in the long run.

"There are a lot of processes being set up, or 'imprinted,' during development, and if something is set up incorrectly, it affects the child's whole life," says Retha Newbold, a biologist and head of the Developmental Endocrinology Section in the Laboratory of Toxicology at the NIEHS. "Children don't have the same protective mechanisms built in that adults do. For example, they don't have the differential metabolic processes to break down some toxicants." Furthermore, she says, "Because a child has a smaller body mass, there may be more of [a toxicant] hitting a smaller target."

Questions abound as to whether toxicants affect children differently than adults, at what levels they cause damage, and if such damage is reversible. Newbold's group and two other groups at the NIEHS are trying to shed light on these issues.

## Estrogens and Children

Newbold concentrates her research on the effects of both estrogens and chemicals in the environment that possess estrogenic activity on fetal and early childhood development. "Everywhere you look, there are either naturally occurring substances or synthetic substances that have estrogenic activity," Newbold says. "And if they have estrogenic activity, they have the potential to have endocrine-disrupting activity."

Newbold has conducted extensive research on the synthetic estrogenic compound diethylstilbestrol (DES), which was developed in 1938. DES was thought to prevent miscarriage and was therefore given to millions of pregnant women from the late 1940s through the 1970s. It was also used as a supplement in cattle and chicken feed for many years.

In 1971, doctors discovered that some of the daughters of women who were given DES developed a rare form of vaginal cancer in their teenage years. Ensuing research revealed that less than 1% of

the daughters developed cancer but that 90–100% of the daughters experienced various reproductive tract problems, including menstrual irregularities and infertility.

In 1975, Newbold and colleagues were the first to discover that DES exposure also affects male offspring in studies of mice. Subsequent human studies found that, indeed, men exposed to DES *in utero* experienced testicular and other reproductive tract abnormalities. More recently, Newbold and colleagues have been investigating the possibility that DES effects could be transmitted to succeeding generations. In 1998, they discovered that increased susceptibility to tumor formation appeared to be transmitted to the granddaughters of female mice exposed to DES while pregnant.

The researchers treated three groups of mice with DES at different stages of development. One group was treated on days 9–16 of gestation, which is a time of major organogenesis. Another group was treated once on day 18 of gestation, just prior to birth, and the third group was treated on days 1–5 after birth.

Fertility was decreased in all groups, as previous research had predicted. The female mice in each group were then bred with control males. The fertility of their offspring was not affected. However, an increased incidence of malignant reproductive tract tumors, including uterine adenocarcinoma, was discovered in all three groups. This indicates that all developmental exposure periods were considered susceptible to the adverse effects of DES. The researchers concluded that, while infertility was not passed on, increased susceptibility to tumor formation appeared to be transmitted through two generations. The results of the study were published in the

September 1998 issue of *Carcinogenesis*.

The finding suggests that DES exposure may cause a defect that is passed on to offspring through multiple generations, says Newbold, and raises the issue that other chemical carcinogenic compounds could have the same effect. Newbold says these findings can be tested in epidemiological studies by following DES-exposed children and grandchildren. It is quite possible that events that lead to tumor formation may begin well before birth and possibly before conception, she says. "Perhaps we need to go back even further in development when looking for a cause and an effect," she says, adding, "Maybe this only happens in mice, but this is an indication that we need to look in humans." She is currently examining the next generation, the great-granddaughters of DES-exposed mice.

Newbold and colleagues are also conducting studies on other estrogenic compounds to determine if there are transgenerational effects. They are also working with the Food and Drug Administration as part of an interagency effort to look at the effects of endocrine disrupting agents in multiple generations. Some of the substances they are investigating are methoxychlor (a pesticide), ethynyl estradiol (a synthetic estrogenic compound used in birth control pills), and genistein (a naturally occurring estrogenic compound found in soy products).

## Pesticides and Children

In 1988, Congress asked the National Academy of Sciences to evaluate the risks posed to infants and children by residual pesticides in the food supply. The academy released a report in 1993, *Pesticides in the Diets of Infants and Children*, that concluded that more information is needed about the

long-term effects of exposure to pesticides during childhood development on the reproductive, immune, and nervous systems.

In response, the U.S. Environmental Protection Agency and the National Toxicology Program (NTP), headquartered at the NIEHS, organized a collaborative research effort in 1994 to examine seven pesticides—methoxychlor, carbaryl, chlorpyrifos, parathion, tebuconazole, atrazine, and trichlorfon. Robert Chapin, a toxicologist in the Reproductive Toxicology Group at the NTP who is heading the research effort, says that each pesticide will take approximately one year to study.



Retha Newbold

Cover credit: The Endocrine Society.

Steve McCaw/Imago Associates, Inc.





Robert Chapin

According to the research protocol, the researchers administer various dose levels of the pesticides to pregnant rats for the week before birth and the week after birth, and to the individual offspring for six weeks after birth. The rats are then divided into groups and tested for reproductive toxicity, immunotoxicity, and neurotoxicity. The researchers also assess the levels of pesticides and metabolites in the breast milk of the exposed mothers.

Methoxychlor, which has been in use for almost 50 years for insect and larval control, was the first pesticide studied. Previous research had indicated that methoxychlor is estrogenic, and that it adversely affects the reproductive system. Because the researchers expected to find changes in reproductive end points after exposure to methoxychlor, they chose to examine it first to serve as a positive control for the study design.

Indeed, the researchers confirmed that methoxychlor is estrogenic. They also found that females are more sensitive than males to methoxychlor, and that low doses, which were previously thought to have no effect, actually do adversely impact the female reproductive system. In males, they found that high doses of methoxychlor affect sperm count while lower doses do not.

At high doses, the researchers observed subtle changes in the behavior of the females, but there were no other apparent behavioral changes, and they found no effects on the immune system. These results were published in the November 1997 issue of *Fundamental and Applied Toxicology*.

The researchers have also completed studies on carbaryl, a short-acting carbamate that is commonly used as a household pesticide, and tebuconazole, a widely used antifungal. The results have been presented at

meetings and are currently being prepared for publication. Chapin says that the researchers found no apparent, consistent effects of carbaryl on the reproductive, immune, or nervous systems. Tebuconazole produced selective effects on learning in rats, which the neurotoxicologists are further exploring. They expect to publish results from these studies in the next year. Current research is focusing on

chlorpyrifos, a relatively long-acting organophosphate. Chapin says the entire research effort should be completed in about four years.

"The wonderful thing about this type of approach is that the whole is greater than the sum of the parts," says Chapin. When all the studies are completed, he says, NTP and EPA researchers and regulators will meet to examine the entire body of data and determine whether patterns exist or if effects are specific to the individual chemicals.

Meanwhile, Chapin and colleagues have also been working with the state of Hawaii to study heptachlor, a pesticide used extensively in pineapple fields in the 1970s. After pineapple harvests, the leaves of the plants are chopped and used as cattle feed. In the early 1980s, large amounts of heptachlor residues were detected in the milk supply from cattle fed with these leaves. Hawaiian children who were briefly exposed to the residues early in life are now reaching adulthood. NTP researchers are currently completing studies on heptachlor and expect to publish results within the next year. The results should help the island state identify possible health effects to be monitored in these young adults.

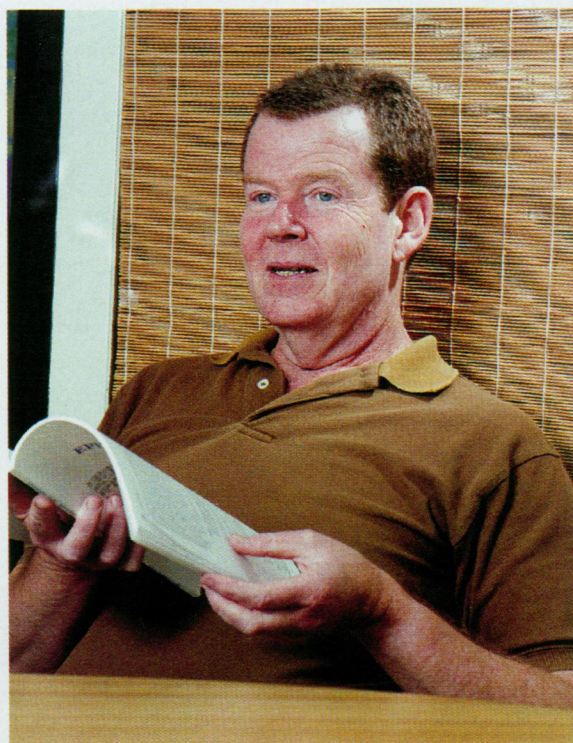
### Pollutants and Children

Walter Rogan, a clinical investigator in the Epidemiology Branch at the NIEHS, has been involved with three major studies examining the effect of pollutant chemicals on the growth and development of children.

In the 1970s, Rogan and colleagues tested the hypothesis that polychlorinated biphenyls (PCBs) and DDE in breast milk might be toxic to infants. They identified 900 children in North Carolina born between 1978 and 1982 and tracked them through puberty. They found that transplacental exposure to PCBs rather than lactational exposure produced small but persistent delays in motor development from birth to age two.

The researchers also showed that DDE at high levels was associated with earlier weaning. They speculated that this was due to the estrogenic activity of DDE, because estrogen inhibits milk production. The researchers later replicated this finding in Mexico. Rogan says this is "the clearest example of environmental estrogens affecting people." He says that the duration of lactation periods is significant in many parts of the world because of the reliance on breast milk to feed infants.

Rogan and colleagues conducted a second study on exposure to PCBs and polychlorinated dibenzofurans following a food poisoning episode in Taiwan in 1979. They examined children who were born up to seven years after the incident, all of whom were transplacentally exposed to the chemicals, which persisted in their mothers' tissues after the women ate contaminated cooking oil. The researchers found that these children experienced ectodermal effects, abnormal behavior, and developmental delay. Rogan says this study, published in the 15



Walter Rogan



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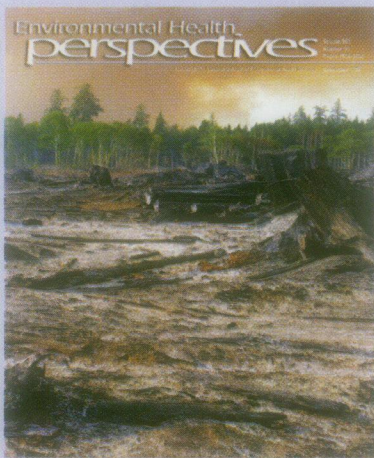
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July 1988 issue of *Science*, was very influential, prompting the state of California to label PCBs as a reproductive hazard for humans.

Rogan is currently project officer for a trial examining treatment of children exposed to lead. Lead has clearly been shown to impair cognitive development in children, producing a dose-related reduction in developmental test scores. An increase in blood lead of 10 µg/dL is associated with a decrease in IQ of 2–3 points. Research has also indicated that exposure to low levels of lead may also affect growth, hearing, behavior, and blood pressure.

In 1991, the Centers for Disease Control and Prevention recommended universal screening of children for blood lead. They recommended chelation therapy for children with levels of 45 µg/dL and higher, no therapy for levels lower than 20 µg/dL, and left the option open for all those in between. Chelation drugs lower blood lead levels by forming a non-covalent bond between the drug and the metal, causing the complex to be more soluble and therefore more readily excreted in urine. It is unclear whether these drugs reverse the effects of the lead exposure because the mechanism by which lead delays development is unknown.

The NIEHS initiated a trial, Treatment of Lead-Exposed Children, to

test the hypothesis that children with moderate blood lead levels treated with succimer, an oral chelating drug, would have higher scores than untreated children on cognitive and behavioral development tests three years after treatment.

Four sites—Baltimore, Maryland; Cincinnati/Columbus, Ohio; Newark, New Jersey; and Philadelphia, Pennsylvania—were selected for the randomized, placebo-controlled, double-blind trial. A cohort of 780 children between the ages of 12 and 33 months with blood levels between 20 and 44 µg/dL qualified for participation in the trial. Investigators conducted baseline age-appropriate psychometric testing (the Bayley Scales of Infant Development II) on the children. They also visited the children's residences and took measures to reduce lead dust in the home.

The children were given up to three 26-day courses of succimer or placebo between August 1994 and January 1997. They were seen at intervals throughout the three years following treatment, and given final IQ tests and other cognitive and behavioral tests, which are ongoing. Rogan says all testing should be complete by the end of this year, and the results should be published in 2000.

Brandy E. Fisher

### Asthma and Allergy Web Site

The NIEHS recently launched a new Web site dedicated to asthma and allergies. The home page, located at <http://www.niehs.nih.gov/airborne/home.htm>, offers links to three major subjects—research studies, allergy prevention, and resources.

The link to research studies provides information about the ongoing asthma studies and programs at the NIEHS. The prevalence of asthma has progressively increased over the past 15 years, now affecting 8–12% of the population and disproportionately affecting children, minorities, and people of lower socioeconomic status.

This page offers links to the five major areas of asthma research being investigated at the NIEHS—primary prevention, secondary prevention, risk assessment, pathogenesis and mechanisms, and genetics. These links offer details about specific NIEHS studies, including the Environmental Intervention in the Primary Prevention of Asthma in Children, the Inner-City Asthma Study, the National Allergen Survey, the Pulmonary Pathobiology Program, and the Environmental Genome Project.

The Allergy Prevention link on the home page provides detailed information on how to reduce exposure to common allergens and asthma irritants such as cigarette smoke, cockroaches, dust mites, food, grass, house dust, mold spores, pet dander, ragweed, and trees. These tips are adapted from a calendar produced by the NIEHS called "Surviving the Seasons: Asthma and Allergy Prevention."

The Resources link on the home page offers links to other organizations and Web sites that provide information on asthma and allergies, including the American Lung Association, the National Allergy Bureau, and the Allergy and Asthma Network.